

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : A61K 31/01, 31/015, 31/045	A1	(11) International Publication Number: WO 94/20080 (43) International Publication Date: 15 September 1994 (15.09.94)
(21) International Application Number: PCT/US94/02340 (22) International Filing Date: 2 March 1994 (02.03.94) (30) Priority Data: 08/025,471 3 March 1993 (03.03.93) US (71) Applicant: THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by THE SECRETARY OF THE DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Box OTT., Bethesda, MD 20892 (US). (72) Inventors: MYERS, Charles, E.; 11111 Ralston Road, Rockville, MD 20852 (US). TREPEL, Jane; 5121 Wisconsin Road, Bethesda, MD 20816 (US). SAUSVILLE, Edward; 1114 Nora Drive, Silver Spring, MD 20904 (US). SAMID, Dvorit; 13 Tapiola Court, Rockville, MD 20852 (US). MILLER, Alexandra; 5306 - 43rd Avenue, Hyattsville, MD 20781 (US). CURT, Gregory; 5018 Cloister Drive, Rockville, MD 20852 (US). (74) Agents: WEBER, Kenneth, A. et al.; Townsend and Townsend Hourie and Crew, Steuart Street Tower, 20th floor, One Market Plaza, San Francisco, CA 94105 (US).		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: USE OF MONOTERPENES, SESQUITERPENES AND DITERPENES FOR THE TREATMENT OF CANCER		
(57) Abstract The invention provides methods of treating cancer including administering an effective amount of selected terpenes to a mammal having the cancer when the cancer is prostate cancer, colon cancer, astrocytoma, or sarcoma. The terpene is selected from the group consisting of a cyclic monoterpene, a noncyclic monoterpene, a noncyclic sesquiterpene and a noncyclic diterpene. The invention also provides a method of sensitizing a cancer to radiation including administering an effective amount of a terpene to a mammal having the cancer wherein the terpene is selected from the group noted above. Additionally, the invention provides methods of inhibiting the growth of cancer cells including applying an effective amount of a selected terpene to the cancer cells which are cells of prostate cancer, colon cancer, osteosarcoma, or glioblastoma.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

Use of monoterpenes, sesquiterpenes and diterpenes for the treatment of cancer

5

BACKGROUND OF THE INVENTION

Treatment of human cancer is an area of clinical medicine which remains fraught with complications and which often presents an array of suboptimal treatment choices. For example, prostate cancer, which is the most common cancer in men and the second leading cause of cancer death in men, can be treated surgically or medically or with a combination of both depending on the state of advance of the cancer. Other than hormonal therapy, no agents are available to treat prostate cancer that are without substantial toxicity. Frequently, cancer occurs in debilitated patients who are either not surgical candidates or who could not tolerate conventionally available chemotherapeutic agents.

Radiation therapy (X-ray treatment) is one modality available to treat selected cancers. It is used at many stages in the treatment of prostate cancer. For example, it can be used as an alternative to surgery to treat localized prostate cancer. X-ray therapy can also be used in metastatic prostate cancer to treat local deposits of tumor that threaten key organs such as the spinal cord. Radiation can be useful to treat a number of cancers in settings such as to attempt the cure of local tumor and the symptomatic management of troublesome metastatic disease.

Osteosarcoma is an example of a cancer primary to the bone but which can metastasize and which remains a major tumor problem in children. A subtype of osteosarcoma, chondrosarcoma, does not metastasize. However, chondrosarcoma often recurs locally after surgical excision and it can require more than surgery alone. Glioblastomas are malignant tumors which do not metastasize generally but which are commonly beyond surgical cure, with median patient survivals of less than one year. A rapidly growing primary tumor that is not surgically curable can cause significant morbidity and

mortality without necessarily metastasizing. In such a case, effective chemotherapy is needed.

More generally, a chemotherapeutic agent which possesses little or no toxicity, which is inexpensive to obtain or manufacture, which is well tolerated by the patient, and which is easily administered would be a desirable addition to the array of therapeutic modalities available to the oncologist. Such a chemotherapeutic agent could find application in treatment of cancers which are metastatic, locally expanding or locally invasive. Additionally, such an agent could be useful if it were to sensitize the cancer to radiation therapy. Thus, the cancer would respond more readily to X-ray treatment.

SUMMARY OF THE INVENTION

The invention provides methods for treating certain cancers comprising administering an effective amount of selected terpenes to a mammal having a cancer susceptible to such treatment. Most preferably, the cancer is an adenocarcinoma of the prostate or colon, a glioblastoma or a sarcoma such as an osteosarcoma. Preferably the administration is accomplished by the oral route and is given one to three times a day. The terpenes are acyclic monoterpenes such as beta-myrcene or citral, cyclic monoterpenes such as limonene, acyclic sesquiterpenes such as farnesol or nerolidol, or acyclic diterpenes such as phytol or geranylgeraniol. Preferably, the mammal treated is a human although the invention can have other applications such as veterinary use.

Additionally, the invention provides a method of sensitizing a cancer to subsequent radiation treatment. This method includes administering an effective amount of a terpene to a mammal having the cancer. The most preferred cancers, terpenes, and administration are as stated above.

The invention also includes a method of inhibiting cancer cell growth comprising applying an effective amount of a terpene to the cancer cells. The most preferred cancers and terpenes are as stated above. If the cells are located in a

mammal, the application is preferably either oral or topical and one to three times a day.

BRIEF DESCRIPTION OF THE DRAWINGS

5 Figure 1 graphs the growth of prostate cancer cells as a percentage of untreated control cells against the concentration of citral in micrograms per milliliter ($\mu\text{g/ml}$) added to the growth medium.

10 Figure 2 graphs the growth of prostate cancer cells as a percentage of untreated control cells against the concentration of nerolidol in $\mu\text{g/ml}$ added to the growth medium.

15 Figure 3 graphs the growth of prostate cancer cells as a percentage of untreated control cells against the concentration of farnesyllic acid in $\mu\text{g/ml}$ added to the growth medium.

 Figure 4 graphs the growth of prostate cancer cells as a percentage of untreated control cells against the concentration of farnesol in $\mu\text{g/ml}$ added to the growth medium.

20 Figure 5 graphs the growth of prostate cancer cells as a percentage of untreated control cells against the concentration of phytol in $\mu\text{g/ml}$ added to the growth medium.

 Figure 6 graphs the growth of prostate cancer cells as a percentage of untreated control cells against the concentration of geranylgeraniol in $\mu\text{g/ml}$ added to the growth medium.

30 Figure 7 displays the growth of human prostate cancer cells as a percentage of untreated control cells when the cancer cells were treated with each of the four named terpenes as compared to farnesol, which is also graphed, in terms of $\mu\text{g/ml}$.

 Figure 8 displays the growth of human prostate cancer cells as a percentage of untreated control cells when the cancer cells were treated with each of the additional four named terpenes as compared to farnesol, which is also graphed, in terms of $\mu\text{g/ml}$.

 Figure 9 displays the growth of human prostate cancer cells as a percentage of untreated control cells when

the cancer cells were treated with each of the additional two named terpenes as compared to farnesol, which is also graphed, in terms of $\mu\text{g/ml}$.

Figure 10 is a radiation dose response curve plotting the surviving fraction of a human osteosarcoma cell line which is radioresistant via activated *ras* oncogene versus the dosage of radiation in gray (Gy), a metric unit for absorbed radiation dose.

Figure 11 is a gel photograph showing DNA ladders.

Figure 12 displays data from an *in vivo* experiment as a dose response curve for phytol treatment of nude mice which were inoculated with human prostate cancer cells. The tumor volume, in cubic millimeters (mm^3), is plotted against the terpene dose in grams per kilogram of body weight per day (gm/kg/day).

Figure 13 displays data from an *in vivo* experiment as a dose response curve for limonene treatment of nude mice which were inoculated with human prostate cancer cells. The tumor volume in mm^3 is plotted against the terpene dose in gm/kg/day .

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The invention includes methods of treating selected cancers comprising administering an effective amount of a terpene to a mammal having the cancer. The cancer is selected from a group consisting of prostate cancer, colon cancer, astrocytoma and sarcoma. The terpene is selected from the group consisting of a cyclic monoterpene, a noncyclic monoterpene, a noncyclic sesquiterpene and a noncyclic diterpene.

Terpenes of the Invention

Terpenes are organic compounds constructed of multiples of the 5-carbon hydrocarbon isoprene unit or 2-methyl-1,3 butadiene ($\text{CH}_2:\text{CMe}\cdot\text{CH}:\text{CH}_2$). Terpenes containing two isoprene units are called monoterpenes, those containing three such units are sesquiterpenes, and those having four isoprene units are diterpenes. Additionally, higher order terpenes exist and there is no set upper limit to how many

isoprene units a terpene may include. For example, natural rubber is a terpene with approximately 60 isoprene units. For information about the structure and synthesis of terpenes, including terpenes of the invention, see *Kirk-Othmer Encyclopedia of Chemical Technology*, Mark, et al., eds., 22:709-762 3d Ed (1983).

The term cyclic means that the compound includes a ring structure such as a six carbon ring. The term noncyclic means that the compound does not include a ring structure but rather is linear in its formulaic depiction. In general, a double bond between the α and β carbons relative to the -OH must be present if an -OH is present. An α carbon is the carbon adjacent to the carbon which bears the functional group. That is, the α carbon is not the carbon attached to the hydroxyl (-OH) group. For example, if the compound is an alcohol, the α carbon is not the carbon to which the -OH group is attached but rather the adjacent carbon. The β carbon is the carbon adjacent to the α carbon moving in the opposite direction from the hydroxyl group. If the functional group is a carboxylic acid group (-COOH), then the α carbon is not the carbon included in the COOH group but rather the adjacent carbon. The β carbon is the carbon adjacent to the α carbon moving in the direction away from or opposite to the functional group which in this example is a COOH group.

The general formula for a cyclic monoterpene is displayed in formula 1. R^1 and R^2 are independently selected from the group including the lower alkyls, the lower alkenes, a hydroxyl, a carboxyl, a carboxylic acid group, and a hydrogen atom. The ring structure preferably includes a double bond, the placement of which depends on the precise location of the R^1 and R^2 groups. The term lower alkyl or lower alkenes refers to structures containing no more than four carbons.

The acyclic monoterpenes are represented generally by formula 3. R^1 , R^2 , R^3 and R_4 are independently selected from the group including the lower alkyls, lower alkenes, a hydroxyl group, a hydrogen atom and a carboxyl or carboxylic acid group. The acyclic monoterpene preferably includes two

or more double bonds. The placement of the double bonds varies with the substitution of the R groups.

The sesquiterpenes are generally represented by formula 6. R^1 , R^2 , R^3 , R^4 , R^5 and R^6 are independently selected from the group consisting of lower alkyls, lower alkenes, hydrogen, hydroxyls, carboxylic acid and hydroxy groups. The structure preferably includes three double bonds, the placement of which varies with the substitutions selected.

The diterpenes are represented generally by formula 9. R^1 , R^2 , R^3 , R^4 , R^5 and R^6 are independently selected from the group including the lower alkyls, lower alkenes, hydrogen hydroxy, hydroxyl oxygen, carboxylic acid groups. The structure preferably includes one or more double bonds, the placement of which may vary.

Preferred Terpenes of the Invention

Drugs useful in the invention have two, three or four isoprene units. Larger structures are insoluble in aqueous solutions and may be solids at room temperature. In the invention, activity of the terpenes increases proceeding from monoterpenes to sesquiterpenes to diterpenes. Examples in increasing order of activity include limonene, farnesol, phytol and geranylgeranol.

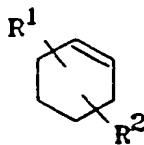
The more active compounds, such as farnesol, phytol and geranylgeranol, all have terminal hydroxy (-OH) groups. Limonene is rapidly metabolized *in vivo* to compounds having terminal hydroxy groups. Also, *in vivo*, these alcohols undergo rapid metabolism to the corresponding aldehydes and carboxylic acids. Such corresponding structures, that is the aldehyde or carboxylic acid, can add to the activity of the parent compound. For instance, farnesyllic acid is an active compound which is identical to farnesol, an alcohol, except that the former terminates in a carboxylic acid group. Citral, a monoterpene having a terminal aldehyde, is also active.

Limonene (formula 2) is an example of a cyclic monoterpene. Examples of acyclic monoterpenes are beta myrcene (formula 4) and citral (formula 5). Examples of sesquiterpenes include farnesol (formula 7), farnesal (the

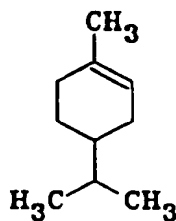
aldehyde of farnesol), farnesylic acid (the carboxylic acid of farnesol) and nerolidol (formula 8). Examples of diterpenes are phytol (formula 11) and geranylgeraniol (formula 10). Limonene and citral are available from Sigma Chemical Company (P.O. Box 14508, St. Louis, MO 63178-9916). Farnesol and phytol are available from Aldrich (1001 W. St. Paul Ave., Milwaukee, WI 53233). Geranylgeraniol is available from American Radiolabeled Chemicals, Inc. The artisan will be aware of modifications which would not significantly reduce the terpene's usefulness. For example, the placement of hydroxy groups or modification to alcohols, aldehydes, and carboxylic acids are noted above. See generally Kirth-Othmer, cited above.

Formula No.:

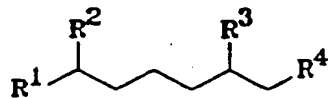
1. Cyclic Monoterpenes



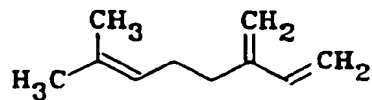
2. d-Limonene



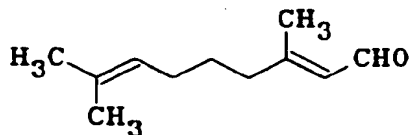
3. Acyclic Monoterpenes



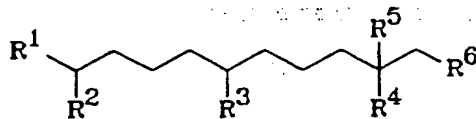
4. Beta myrcene



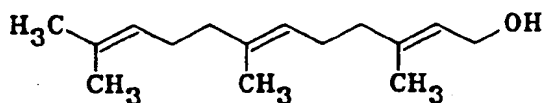
5. Citral



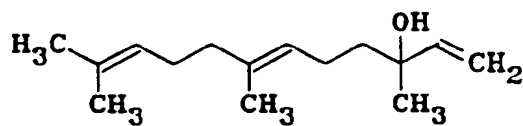
6. Sesquiterpenes



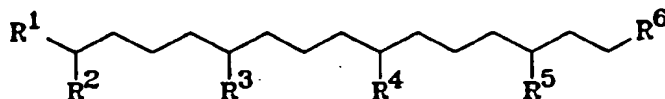
7. Trans, trans farnesol ("t,t farnesol")



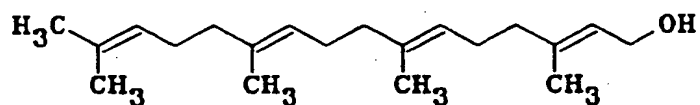
8. Nerolidol



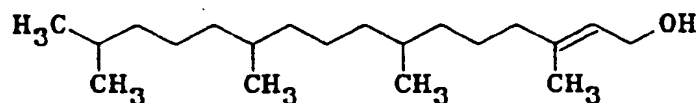
9. Diterpenes



10. Geranylgeraniol



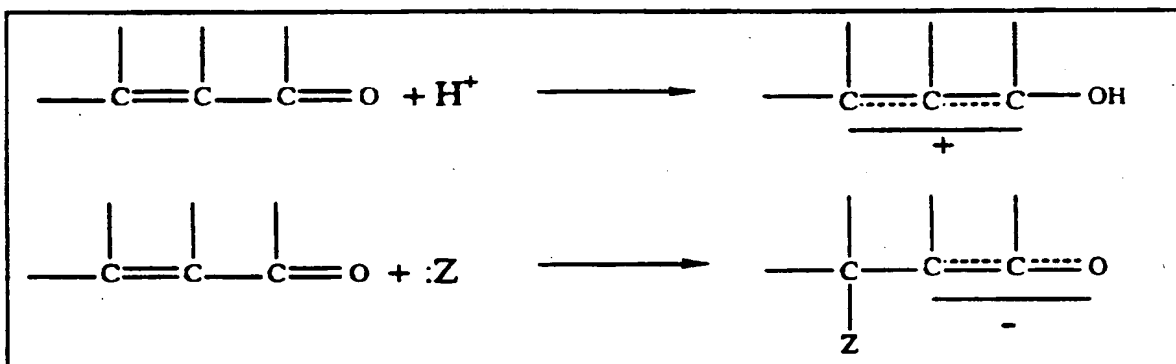
11. Phytol



Analog, Homologs and Derivatives

The preferred compounds are farnesol, geranylgeranol and the geranylgeranol analog, phytol. These compounds are oxidized enzymatically to the corresponding aldehyde and carboxylic acids. These carbonyl metabolites are alpha, beta-unsaturated compounds and as such have the capacity to participate in a range of nucleophilic and electrophilic addition reactions.

10 Nucleophilic and Electrophilic Addition and Alpha, Beta Unsaturated Carbonyls:



Note that a similar reaction occurs with carboxylic acids, which also contain carbonyls. Z may be any naturally occurring amino acid able to act as a nucleophil.

Geranylgeranyl- and farnesyl pyrophosphate are endogenous metabolites found in all mammalian cells that undergo a wide range of transferase reactions that all have in common the fact that the pyrophosphate acts as a leaving group and the alkene forms a carbocation. The latter alkylates the other substrate in the enzyme reaction. For those agents not convertible to alkylating moieties, such as an alpha, beta unsaturated carbonyl, the effect would be a reversible inhibition. For the alkylating analogs, the potential exists for irreversible active-site directed alkylation. Thus, the invention includes any terpene which is 10 to 20 carbons in length and which either terminates in an alpha, beta unsaturated carbonyl or which is converted to such a metabolite *in vivo*.

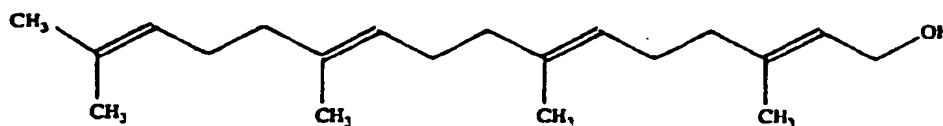
For instance, there is a series of acyclic diterpenes closely related to geranylgeraniol that may be used in the invention. At present, only phytol and geranylgeraniol are commercially available. The others are isolated from their natural sources. Geranylgeraniol is naturally available as an isolate of linseed oil and cedrela toona wood. Phytol can be isolated from green plants. 9-hydroxygeranylgeraniol can be isolated from brown seaweed, geranylinalool from jasmine oil and Norwegian spruce, and geranylcitronellol from bumblebee secretion.

The pyrophosphate not only acts as a leaving group, but the free pyrophosphate anion released acts as an ion-pair for the terpene carbocation. Thus the terpenes of the invention further include attachment of any substituent to C1 of the terpene that endows the compound with alkylating activity. Also included are terpenes having any leaving group other than pyrophosphate. However, this last group may be too unstable for use as a drug in humans and might cross cell membranes poorly because of its charge. These other leaving groups may have a structure that might interfere with the normal function of the free pyrophosphate anion. In general, preferable analogs are uncharged at physiologic pH, so as to promote uptake by tumor cells.

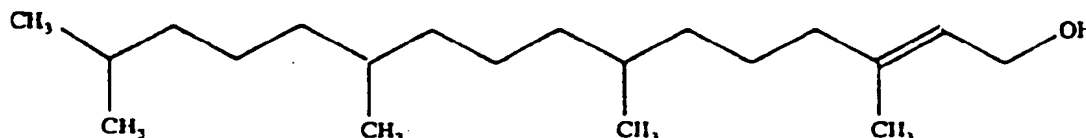
Geranylgeraniol, phytol and some diterpene analogs

Formula No.:

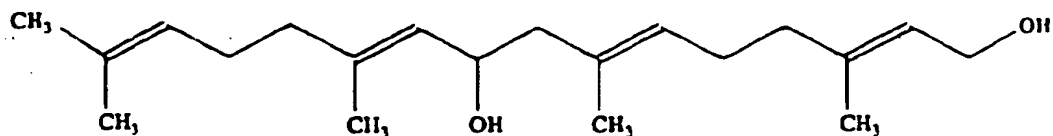
12. Geranylgeraniol (Linseed Oil; Cedrela toona wood)



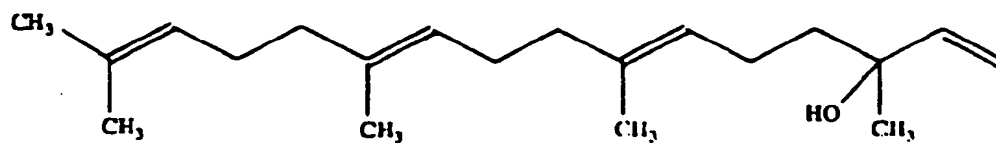
13. Phytol (All green plants)



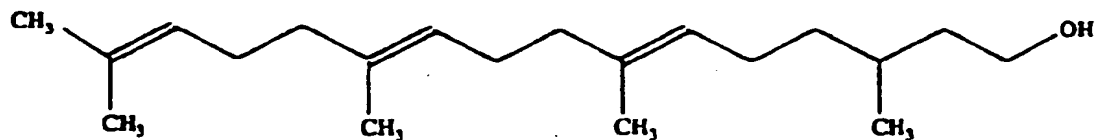
14. 9-Hydroxygeranylgeraniol (Brown Seaweed)



15. Geranyllinalool (Jasmine Oil; Norwegian spruce)

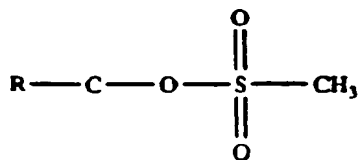


16. Geranylcitronellol (Bumblebee secretion)

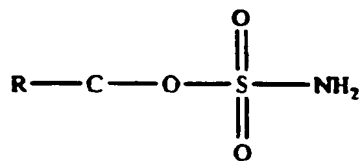
Sulfonic Acid Derivatives

Formula No.:

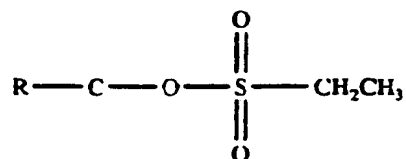
17. Methanesulfonate



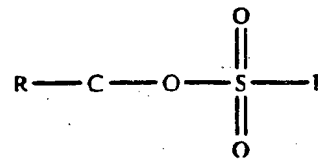
18. Sulfonamide



19. Ethanesulfonate

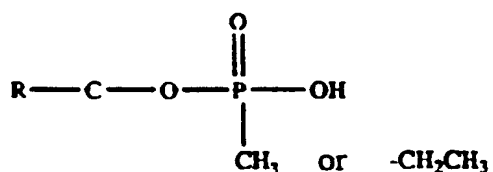


20. Sulfonylfluoride

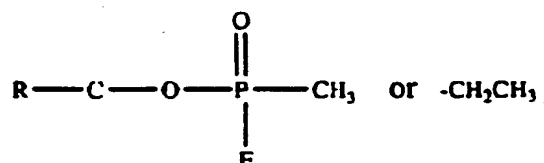
Phosphate analogs

Formula No.:

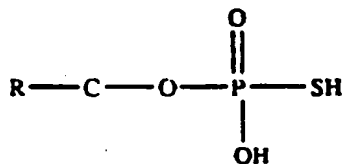
21. Methyl or ethyl phosphate



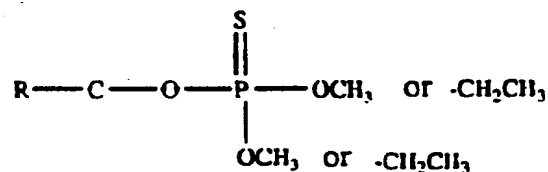
22. Methyl or ethyl, fluoro



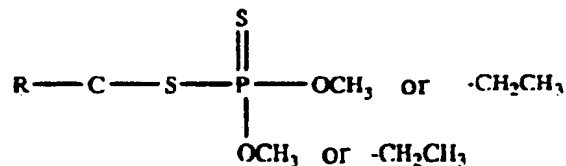
23. Phosphorothioate



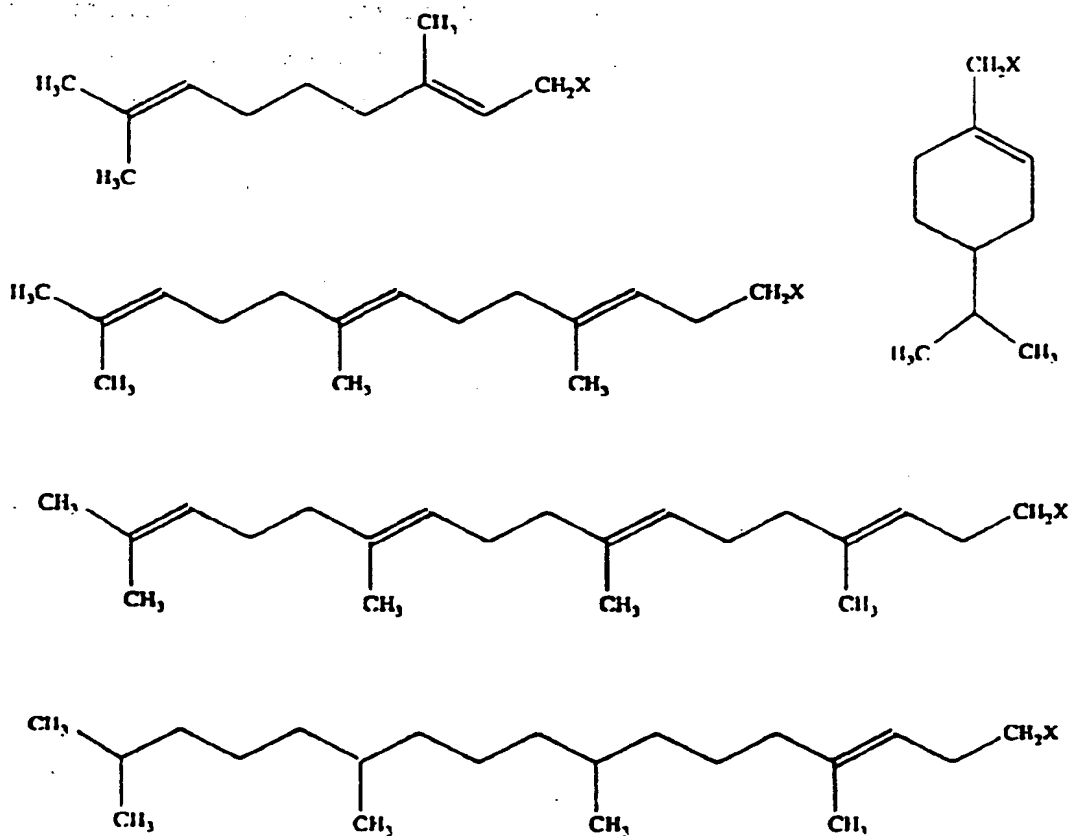
24. Dimethyl or methyl, ethyl or diethyl, phosphorothioate



25. Dimethyl or ethyl, methyl or diethyl, phosphorodithioate



R Groups for derivatives and analogs numbers 17-25. The Site of Attachment is at X.



Cancer Therapy

Generally, prostate cancers and colon cancers are adenocarcinomas. However, in roughly five percent of the cases either of these cancers are some other type. An example of an astrocytoma is a glioblastoma. Examples of sarcomas include osteosarcomas, fibrosarcomas, and rhabdomyosarcomas. The cancer to be treated can be metastatic, locally expanding or locally invasive.

The administration of the terpene can be accomplished by any of a number of routes but it is usually either oral or topical with the oral route preferred. The oral route is preferred. The term "oral route" means that the patient would swallow the drug. The term also includes gavage, which is a preferred delivery in nonhuman animals.

Preferably the mammal treated is a human. For humans, the effective amount of terpene is selected from a range of from about 9 milligrams per kilogram of body weight of the mammal per day (mg/kg/day) to about 0.10 grams per kilogram of body of the mammal per day (gm/kg/day). A more preferred effective amount is selected from the range of about 0.02 to about 0.15 gm/kg/day. A single daily administration is typical, but the dose can be given two, three or more times a day. The terpene is usually administered on a long-term basis or chronically for weeks, months, or years.

The terpenes of the invention can be stored at room temperature, preferably protected from bright light and away from direct heat. The pleasant taste and odor of these terpenes permit their use undiluted, mixed with liquid or given with food. Juices, dairy products, and mashed fruits make particularly suitable vehicles for oral consumption. Alternatively, the terpene can be presented in encapsulated form, in a syrup, a gel, a lozenge or other conventional means for delivering oral medication. Additionally, the terpene can be diluted to a preselected concentration in a liquid such as a saccharide based syrup. The patient is given (or instructed to take) a specified volume, such as a tablespoonful, which can be taken alone or mixed in liquid or semi-liquid comestibles as noted above.

Sensitizing a Cancer to Radiation

The invention also includes a method of sensitizing a cancer to radiation comprising administering an effective amount of a chosen terpene to a mammal having the cancer. The terpene is selected from the group consisting of a cyclic monoterpene, a noncyclic monoterpene, a noncyclic sesquiterpene, and a noncyclic diterpene. The cancer is preferably either prostatic or colonic. The cancer may be selected from a group of adenocarcinoma, astrocytoma, and sarcoma. The adenocarcinoma is preferably either prostatic or colonic. The astrocytoma is preferably a glioblastoma. The sarcoma is preferably an osteosarcoma, a fibrosarcoma, or a rhabdomyosarcoma.

The administration is either oral or topical with the oral route preferred. Preferably the mammal is a human and the administration is once to three times a day. The cancer can be either locally expanding, locally invasive or metastatic. The effective amount for humans is selected from a range of from 9 mg/kg/day to about 0.10 gm/kg/day. More preferably, the effect of the amount is selected from a range of about 0.02 to about 0.15 gm/kg/day. The acyclic monoterpene is preferably either beta myrcene or citral. The cyclic monoterpene is preferably limonene. The sesquiterpene is preferably selected from a group consisting of farnesol, farnesal, farnesylic acid and nerolidol. The diterpene is preferably selected from a group consisting of phytol, phytal, phytylic acid, geranylgeraniol, geranylgeranial, and geranylgeranylic acid.

Inhibition of Cancer Cell Growth

The invention also includes a method of inhibiting the growth of cancer cells comprising applying an effective amount of a selected terpene to the cancer cells. The cancer cells are chosen from the group consisting of prostate cancer, colon cancer, osteosarcoma and glioblastoma. The terpene is selected from a group consisting of beta myrcene, citral, limonene, farnesol, farnesal, farnesylic acid and nerolidol, phytol, phytal, phytylic acid, geranylgeraniol, geranylgeranial, and geranylgeranylic acid.

The application of the terpene to the cells is either direct or indirect. The cells can be located in a mammal. When the cells are located in a mammal the application is usually indirect and preferably oral. Direct application can be made topically. The mammal is preferably a human and the application is preferably once to three times a day. The effective amount for humans is selected from a range from about 9 mg/kg/day to about 0.10 gm/kg/day. A more preferred effective range is from about 0.02 to about 0.15 gm/kg/day.

Definitions

The term "effective amount" means a dosage sufficient to produce a desired result. The desired result can be subjective or objective improvement in the recipient of the dosage, a decrease in tumor size, a decrease in the rate of growth of cancer cells, and/or an enhanced sensitivity of cancer cells to radiation.

The terms "treating cancer", "therapy" and the like mean generally any improvement in the mammal having the cancer wherein the improvement can be ascribed to treatment with the terpene. The improvement can be either subjective or objective. For example, if the mammal is human, the patient may note improved vigor or vitality or decreased pain as subjective symptoms of improvement or response to therapy. Alternatively, the clinician may notice decrease in tumor size or tumor burden based on physical exam, laboratory parameters, tumor markers or radiographic findings.

Some laboratory signs that the clinician may observe for response to therapy include normalization of tests such as white blood cell count, red blood cell count, platelet count, erythrocyte sedimentation rate, and various enzyme levels such as transaminases and hydrogenases. Additionally, the clinician may observe a decrease in a detectable tumor marker such as prostatic specific antigen (PSA) or chorio embryonic antigen (CEA). Alternatively, other tests can be used to evaluate objective improvement such as sonograms, nuclear magnetic resonance testing and positron emissions testing.

"Inhibiting the growth of cancer cells" can be evaluated by any accepted method of measuring whether growth of the cancer cells has been slowed or diminished. This includes direct observation and indirect evaluation such as subjective symptoms or objective signs as discussed above.

The term "sensitizing a cancer to radiation" means that as a result of treatment with the terpene, the cancer is more likely to respond to radiation exposure than it would have been without treatment with the terpene. The term "respond to radiation" means that the cancer is expected to either regress or slow its growth subsequent to radiation therapy.

In referring to specific terpenes the corresponding alcohol, aldehyde and carboxylic acid are included. Such derivatives are sometimes referred to as "terpenoids." Additionally, active metabolites of the terpenes are included. For instance, the terms "limonene, farnesol, phytol and geranylgeraniol" include the active metabolites as well as the administered parent compounds or pro-drugs.

Dosage Conversions

In living animals, metabolic rate decreases as body size increases. For drugs that undergo metabolism or act by altering a metabolic event, drug dosing must take this into consideration. Body surface area correlates better with metabolic rate than does body weight. For this reason, it is best to use body surface area to convert dosing in one species to that of another. See Freireich, et al., *Cancer Chemotherapies* 50(4)219-244 (1966). Freireich provided correlation factors using body surface area to convert mouse doses to that of other species. A rat would get about one-half of the mouse dose; a monkey which weighs about three kilograms would get about one-fourth of the mouse dose; a dog which weighs about 8 kilograms would get one-sixth of the mouse dose and a human would get about one-twelfth of the mouse dose. Thus, a 14 gram dose in a mouse correlates to slightly over one gram in a human and a 126 gram dose in a mouse correlates to about eleven grams in a human. The mouse dose of the invention is selected from a range of from about

100 mg/kg/day to about 2 gm/kg/day. A more preferred range for the mouse is from about 0.2 to about 1.8 mg/kg/day.

The following examples are illustrative. The invention is not limited by the specification and illustrations, but rather by the appended claims.

Examples

Example 1: *In vitro* antitumor activity of selected terpenes against the PC3 human prostate carcinoma.

A human prostate carcinoma cell line, PC3, was obtained from the American Tissue Culture Collection, Accession No. CRL 1435. The PC3 cells were cultured in RPMI 1640 medium enriched with 10% fetal calf serum (FCS). The medium and the FCS are both available from GIBCO BRL Life Technologies, Inc., 8400 Helgeman Lane, Gaithersburg, MD 20877.

Terpenes were exposed to the PC3 cells as follows. On day 0, seed PC3 cells at about 1,000 cells per well were placed in each well of a 96-well tissue culture plate along with 0.1 milliliter (ml) of culture medium. On day 1, various concentrations of each of the selected terpenes were added. A serial dilution of each terpene was made in dimethyl sulfoxide (DMSO). All of the initial concentrations were about 200 times the desired concentration. Each dose was further diluted 100 times with culture medium. 0.1 ml of each dose was added per well to the tissue culture plate. The plates were incubated at 37° Celsius (C) under 5% carbon dioxide (CO₂) for seven days. The results are depicted graphically in Figs. 1-9 and some of the concentrations which inhibited cell growth to about 50% of the control growth (IC₅₀) are shown in Table 1.

Table 1 *In vitro* antitumor activity of selected terpenes against the PC3 human prostate carcinoma (Seven day exposure)

Terpene	IC50 in μ M	μ g/ml
1. d-Limonene	217	30
2. beta-myrcene	147	21
3. citral	17	3

20

4. trans, trans-farnesol	85.6	19
5. farnesyllic acid	63.2	15
6. nerolidol	90.1	20
7. phytol	34.3	10
8. geranylgeraniol	10.5	3.0

Figures 1-6 show that citral, nerolidol, farnesyllic acid, trans-trans-farnesol, phytol, and geranylgeraniol all demonstrated favorable activity against the human prostate cancer cells.

Figures 7-9 indicate that some additional terpenes were less active than farnesol, which was used as a standard in these figures. The less active compounds include dihydromyrcenol, 4-isopropylbenzaldehyde, 4-isopropylbenzylalcohol, 4-isopropylbenzoic acid, perillartine, citronellic acid, d-citronellol, citronellal, nerol and 16-hydroxyhexadecanoic acid.

Example 2: Effect of limonene on various tumor cell proliferations.

Limonene (Sigma) was evaluated for its effect on proliferation of various tumor cell lines. The cell lines included U87 and A172 (both are glioblastomas), HOS (a low ras osteosarcoma), AD5 (a high ras osteosarcoma), and HT29 (a high ras colon carcinoma). An activated or high ras means that the ras oncogene has mutated to a form which can transform normal cells to malignant cells. The glioblastoma cells were grown in RPMI 1640 culture medium and the other three cell lines were grown in Dulbecco's Modified Eagle's Medium (DMEM, available from GIBCO).

Limonene was added to the growth medium, which was supplemented with 10% FCS, and incubated at 37°C for 24 hours. The medium was added to human tumor cells plated the previous day in 60 millimeter (mm) tissue culture (TC) dishes. After three to four days of continuous treatment, the cells were detached by trypsinization and counted using a hemocytometer (Hy-Lite Counting Chamber from Fisher Scientific, 711 Forbes Ave., Pittsburgh, PA 15219-4785). Cell viability was

determined by a trypan blue exclusion method as described in detail in the manufacturer's instructions. As shown in Table 2, limonene provided a favorable IC₅₀ as measured in millimoles (mM).

Table 2: Effect of limonene on tumor cell proliferation

Cell line	Growth Medium	Limonene IC ₅₀ * (mM)
U87 glioblastoma	RPMI 1640	2.1 ± 0.4
A172 glioblastoma	RPMI 1640	1.5 ± 0.5
HOS osteosarcoma (low ras)	DMEM	3.0 ± 0.3
AD5 osteosarcoma (high ras)	DMEM	4.8 ± 0.6
HT29 colon carcinoma (high ras)	DMEM	4.5 ± 0.5

*cell viability >95%, meaning that of the approximately 50% of cells which survived, >95% were viable.

Example 3: The effect of limonene on radiation sensitivity of tumor cells.

Radiation dosing in humans can be as low as 100 to 200 rads for a total body radiation therapy. The word "rad" refers to an absorbed radiation dose equal to 0.01 Joules (J)/kg of the absorbing medium such as tissue. For local radiation therapy isolating a tumor deposit, dosage can range from about 2,000 to 4,000 rads for lymphomas and up to about 6,000 to 7,000 rads for tumors such as prostate cancer that are normally radiation resistant.

An activated ras oncogene makes tumor cells resistant to radiation therapy. The AD5 human osteosarcoma cell line is an example of such a cell line resistant to radiation by virtue of an activated ras oncogene. AD5 cells were cultivated as described in Example 2. Limonene was prepared in various concentrations also as described in Example 2. Fig. 10 shows the radiation dose response of this cell line in the presence (diamonds) and in the absence (open boxes) of 3 mM limonene. Limonene alters both the shoulder and the dose (D₀) of the radiation dose response curve. This

dose refers to the dose that reduces the number of surviving cells to about 37% of the control cells. Gy (gray) is an international metric unit for an absorbed dose of radiation. One Gy equals one Joule/kg.

Example 4: Apoptosis and nuclear fragmentation.

Drugs can be cytostatic in that they suppress tumor growth, but do not kill. Other drugs kill tumor cells through either necrosis or apoptosis. The latter is also called programmed cell death and is a physiologic means of cell death. For example, lymphocytes exposed to glucocorticoids die by apoptosis. Involution of hormone sensitive tissue such as breast and prostate that occurs when the trophic hormone is removed occurs via apoptosis.

The invention examined the effect on prostate tumor cells of treatment with limonene, farnesol and phytol. With each of these compounds, tumor cell death (not cytostasis) was observed. Also, in each case, the mode of cell death was apoptosis, not necrosis. The classic signature of apoptosis is the cleavage of nuclear DNA into nucleosomal subunits. On gels, this gives rise to the appearance of a ladder as nucleosomal units are sequentially cleaved from the DNA. With each of these drugs, a classic DNA ladder indicative of apoptosis has been noted.

After drug exposure, cells were lysed and the high molecular DNA removed by centrifugation. The aqueous phase was treated with proteinase K to digest proteins. After a phenol/chloroform extraction, the DNA was precipitated with salt and ethanol. The pellet was dissolved in deionized water and treated with 500 µg/ml RNase A. The DNA was run on a 2% agarose minigel. Figure 11 is a gel photograph showing classic DNA ladders for farnesol (F) and limonene (L). Cell death was verified by the demonstration of DNA fragmentation as represented by the ladder configurations on the gel.

Example 5: In vivo antitumor activity of d-limonene against PC3 human prostate carcinoma in nude mice.

Nude mice were obtained from Cancer Research Facility in Frederick, Maryland. The strain name is Athymic MCR-nude. PC3 cells were transplanted into both the right and left flanks of nude mice and allowed to grow until the tumor size reached the volume of approximately 200-300 cubic mm (mm^3). At this time, five mice bearing ten tumors total were allocated to each group.

The drug or vehicle alone were administered by gavage once daily Monday through Friday for two weeks. The control group received olive oil at a dose of 0.1 cubic centimeters (cc) per ten grams (gm) of body weight. Other groups received the same volume of olive oil with limonene added to give a daily dose of limonene of 0.1, 1.0 or 2.0 milligrams of drug per kilogram of body weight (mg/kg). Both body weight and tumor volume were measured daily.

The animals appeared to tolerate the drug well and even at the highest dose level there was not a statistically significant loss in weight over the course of the experiment. The rate of tumor growth was the same in the control group and those treated with 0.1 and 1.0 gm/kg. However, the rate of tumor growth in the animals receiving 2.0 gm/kg was approximately half of that in the control group. This result was highly significant with low p values for the differences in tumor volume ($p=0.031$) and rate of tumor growth ($p=0.0022$) by day 14 in the control as compared to the animals receiving limonene at 2 gm/kg. See Fig. 12 which plots the tumor volume in mm^3 against the dose of limonene in gm/kg/day.

Example 6: In vivo antitumor activity of phytol against PC3 human prostate carcinoma in nude mice.

The experimental procedure of Example 5 was followed except that phytol was used in place of limonene. The animals tolerated the phytol well. The results are depicted in Fig. 13 which plots the percent change of tumor volume versus the number of days after inoculation of the nude mice with TC3 tumor cells. The treated animals (open squares) produced a lower tumor volume as compared to the control animals (closed

diamonds). During the treatment period (indicated by a bar) the treated animals showed negligible tumor production.

The equation is obtained by applying a least squares regression of the equation $y = b + mx$ to the data. The resulting equation is the best fit to the data. In this example, "y" represents the percentage change in tumor volume and "x" represents the number of days. The γ value represents the "goodness" or satisfaction of the fit. If $\gamma = 1$, then the fit is perfect. If $\gamma = 0$, then there is no correlation. In this case, $\gamma = 0.985$, demonstrating a nearly perfect fit.

Example 7: A clinical trial.

The patient selected for treatment is a man having prostate adenocarcinoma and who is not a candidate for surgical treatment. Because the patient weighs about 70 kilograms, the dosage selected can range from about 1 gram to about 11 grams total per day (about one-twelfth the mouse dose of 0.2 to 1.8 gm/kg/day) orally. This dose corresponds to about 2 ml to about 14 ml of undiluted drug. The patient is started on an intermediate dosage of 2 grams orally three times a day. The terpene is phytol.

The patient is monitored for symptoms and signs of drug toxicity such as loss of appetite. If toxicity is noted, the drug administration is suspended. Drug administration is reinstituted when the patient recovers but at a dosage reduced by about 25%. The phytol is administered on a long-term or chronic basis. Although the patient is monitored carefully throughout the entire treatment course, monitoring is most intensive during the first month of therapy.

Preferably, the response is arrest of tumor growth. Various means of assessing tumor growth are available including physical examination, determination of levels of prostate specific antigen, sonographic studies and radiographic evaluations. Additionally, parameters are monitored to assess the patient's general tolerance of the drug and well being. For example, blood or serum tests for complete blood counts, electrolytes, minerals, enzymes and various other factors may be monitored.

WHAT IS CLAIMED IS:

1. A method of treating cancer comprising administering an effective amount of a terpene to a mammal having the cancer, said cancer selected from a group consisting of prostate cancer, colon cancer, astrocytoma, and sarcoma, and said terpene selected from the group consisting of a cyclic monoterpene, a noncyclic monoterpene, a noncyclic sesquiterpene, and a noncyclic diterpene.
2. The method of claim 1 wherein the astrocytoma is a glioblastoma.
3. The method of claim 1 wherein the sarcoma is selected from a group consisting of an osteosarcoma, a fibrosarcoma, and a rhabdomyosarcoma.
4. The method of claim 1 wherein the cancer is metastatic.
5. The method of claim 1 wherein the administration is either oral or topical.
6. The method of claim 1 wherein the mammal is a human.
7. The method of claim 6 wherein the effective amount is selected from a range of from about 9 mg/kg/day to about 0.10 gm/kg/day.
8. The method of claim 7 wherein the effective amount is selected from a range of from about 0.02 to about 1.15 mg/kg/day.
9. The method of claim 1 wherein the acyclic monoterpene is either beta myrcene or citral.

10. The method of claim 1 wherein the cyclic monoterpene is limonene.

11. The method of claim 1 wherein the sesquiterpene is selected from a group consisting of farnesol, farnesal, farnesylic acid and nerolidol.

12. The method of claim 1 wherein the diterpene is either phytol or geranylgeraniol.

13. A method of sensitizing a cancer to radiation comprising administering an effective amount of a terpene to a mammal having the cancer, said terpene selected from the group consisting of a cyclic monoterpene, a noncyclic monoterpene, a noncyclic sesquiterpene, and a noncyclic diterpene.

14. The method of claim 13 wherein the cancer is either prostatic or colonic.

15. The method of claim 13 wherein the cancer is selected from a group consisting of adenocarcinoma, astrocytoma, and sarcoma.

16. The method of claim 15 wherein the adenocarcinoma is either prostatic or colonic.

17. The method of claim 15 wherein the astrocytoma is a glioblastoma.

18. The method of claim 15 wherein the sarcoma is selected from a group consisting of an osteosarcoma, a fibrosarcoma, and a rhabdomyosarcoma.

19. The method of claim 13 wherein the administration is either oral or topical.

20. The method of claim 13 wherein the mammal is a human.

21. The method of claim 20 wherein the effective amount is selected from a range of from about 9 mg/kg/day to about 0.10 gm/kg/day.

22. The method of claim 21 wherein the effective amount is selected from a range of from about 0.02 to about 0.15 gm/kg/day.

23. The method of claim 13 wherein the acyclic monoterpene is either beta myrcene or citral.

24. The method of claim 13 wherein the cyclic monoterpene is limonene.

25. The method of claim 13 wherein the sesquiterpene is selected from a group consisting of farnesol, farnesal, farnesylic acid and nerolidol.

26. The method of claim 13 wherein the diterpene is selected from a group consisting of phytol, phytal, phytylic acid, geranylgeraniol, geranylgeranial, and geranylgeranylic acid.

27. The method of claim 13 wherein the cancer is metastatic.

28. A method of inhibiting the growth of cancer cells comprising applying an effective amount of a terpene to the cancer cells, said cancer cells selected from the group consisting of prostate cancer, colon cancer, osteosarcoma, and glioblastoma and said terpene selected from a group consisting of beta myrcene, citral, limonene, farnesol, farnesal, farnesylic acid, nerolidol, phytol, phytal, phytylic acid, geranylgeraniol, geranylgeranial, and geranylgeranylic acid.

29. The method of claim 28 wherein the application is indirect.

30. The method of claim 29 wherein the cancer cells are located in a mammal and the application is oral.

31. The method of claim 30 wherein the mammal is a human.

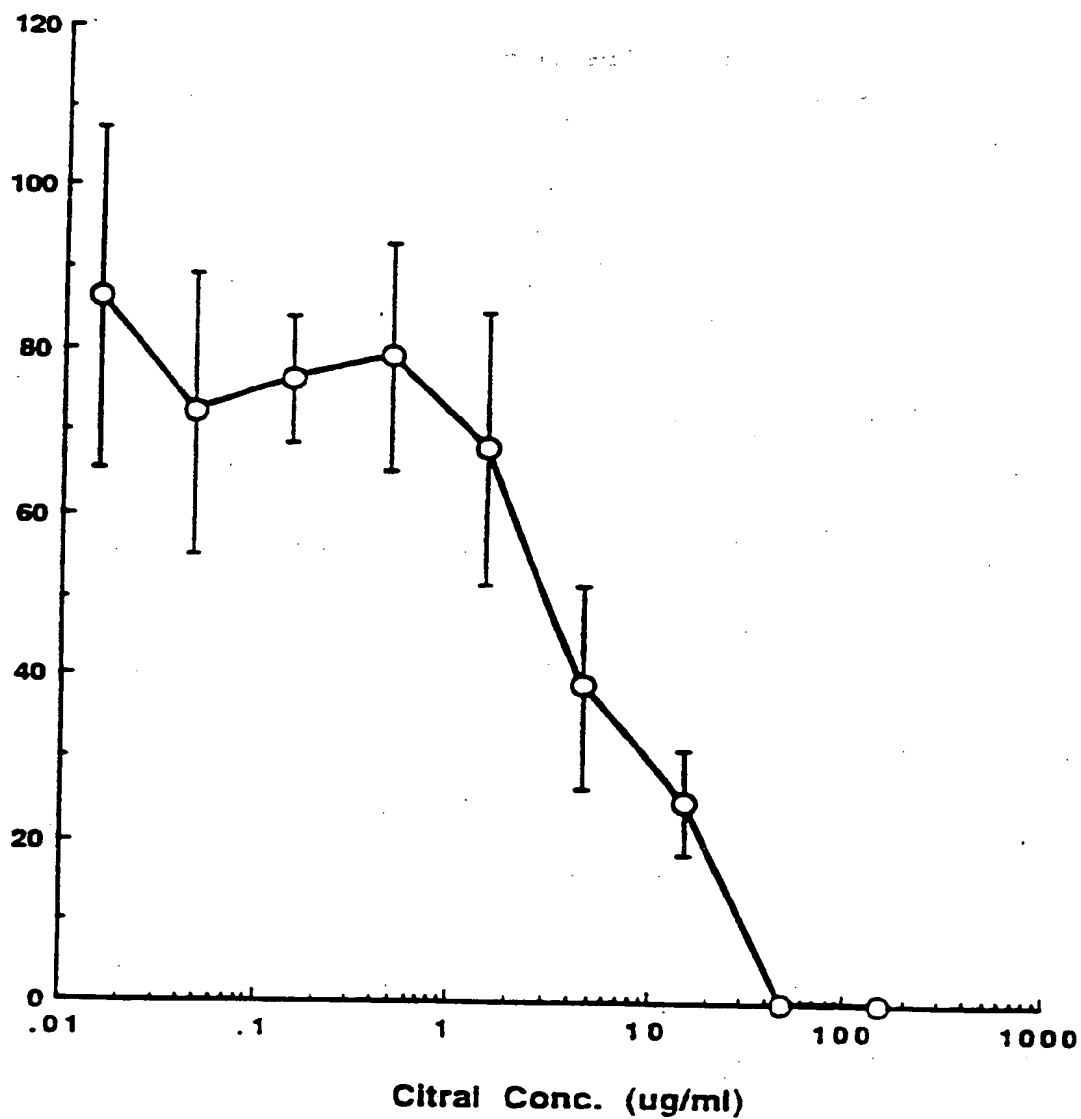
32. The method of claim 31 wherein the effective amount is selected from a range of from about 9 mg/kg/day to about 0.10 gm/kg/day.

33. The method of claim 32 wherein the effective amount is selected from a range of from about 0.02 to about 0.15 gm/kg/day.

34. The method of claim 30 wherein the application is made once a day.

- 1 / 13

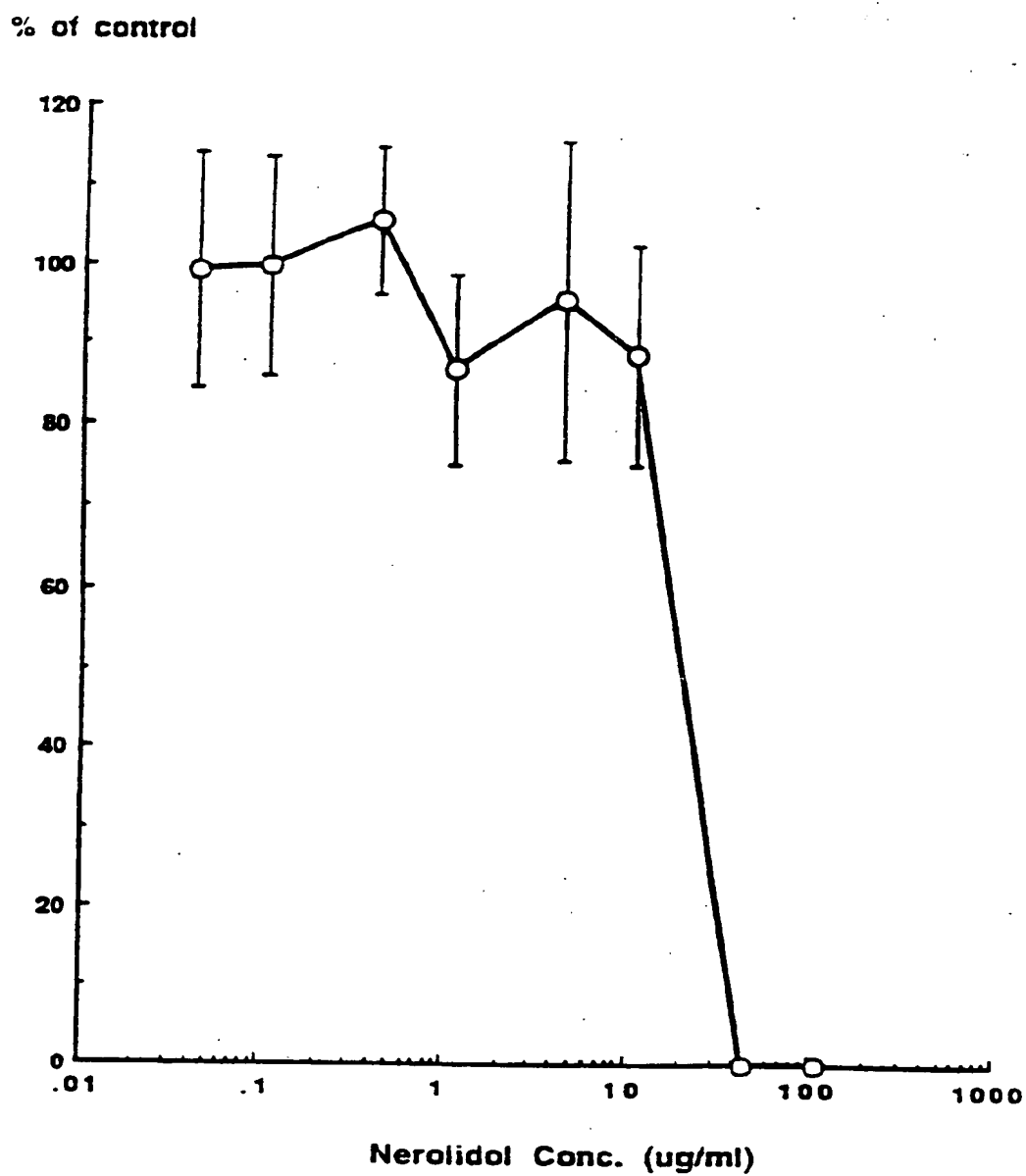
% of control



Effect of Citral on PC 3 Growth

FIG. 1

- 2 / 13



Effect of Nerolidol on PC 3 Growth

FIG. 2

- 3 / 13

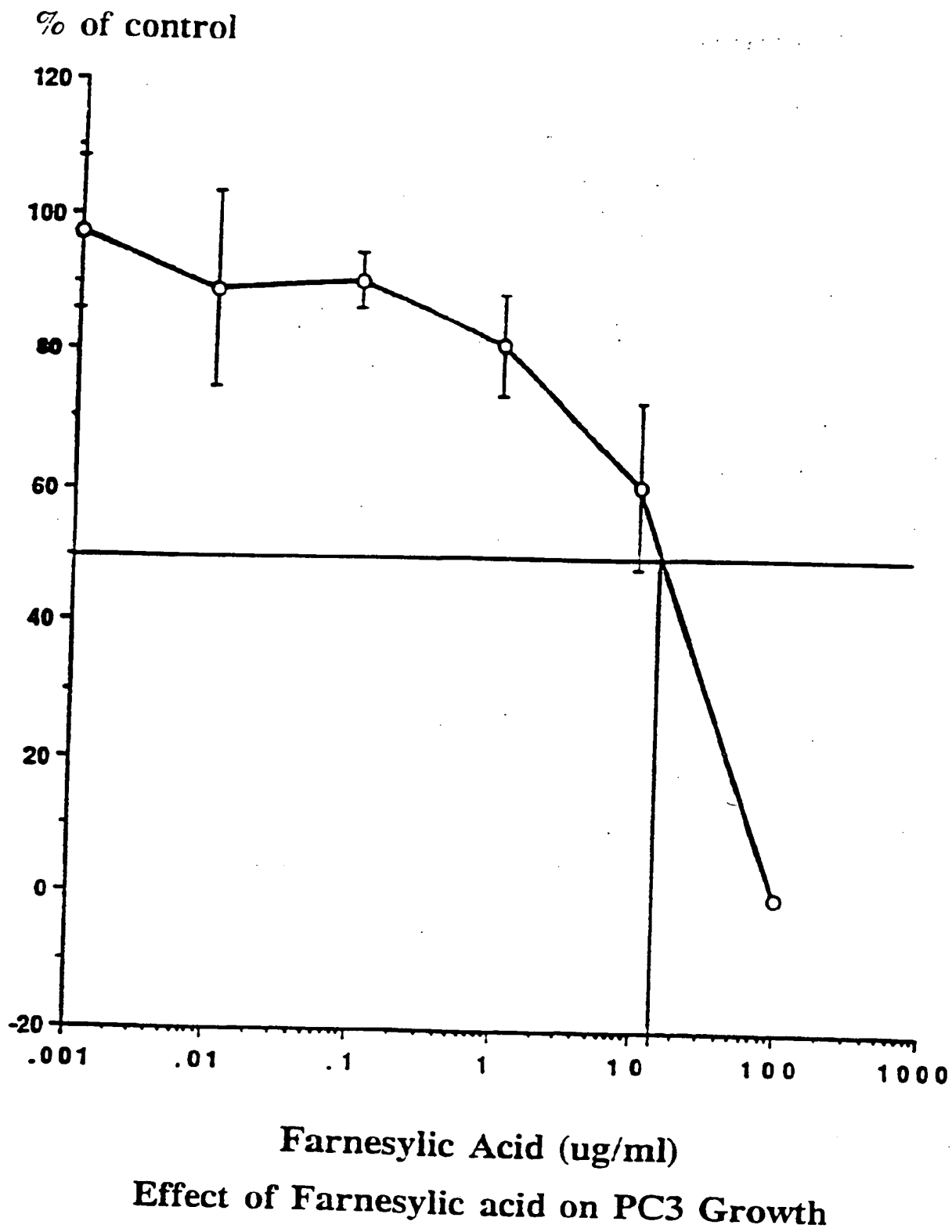
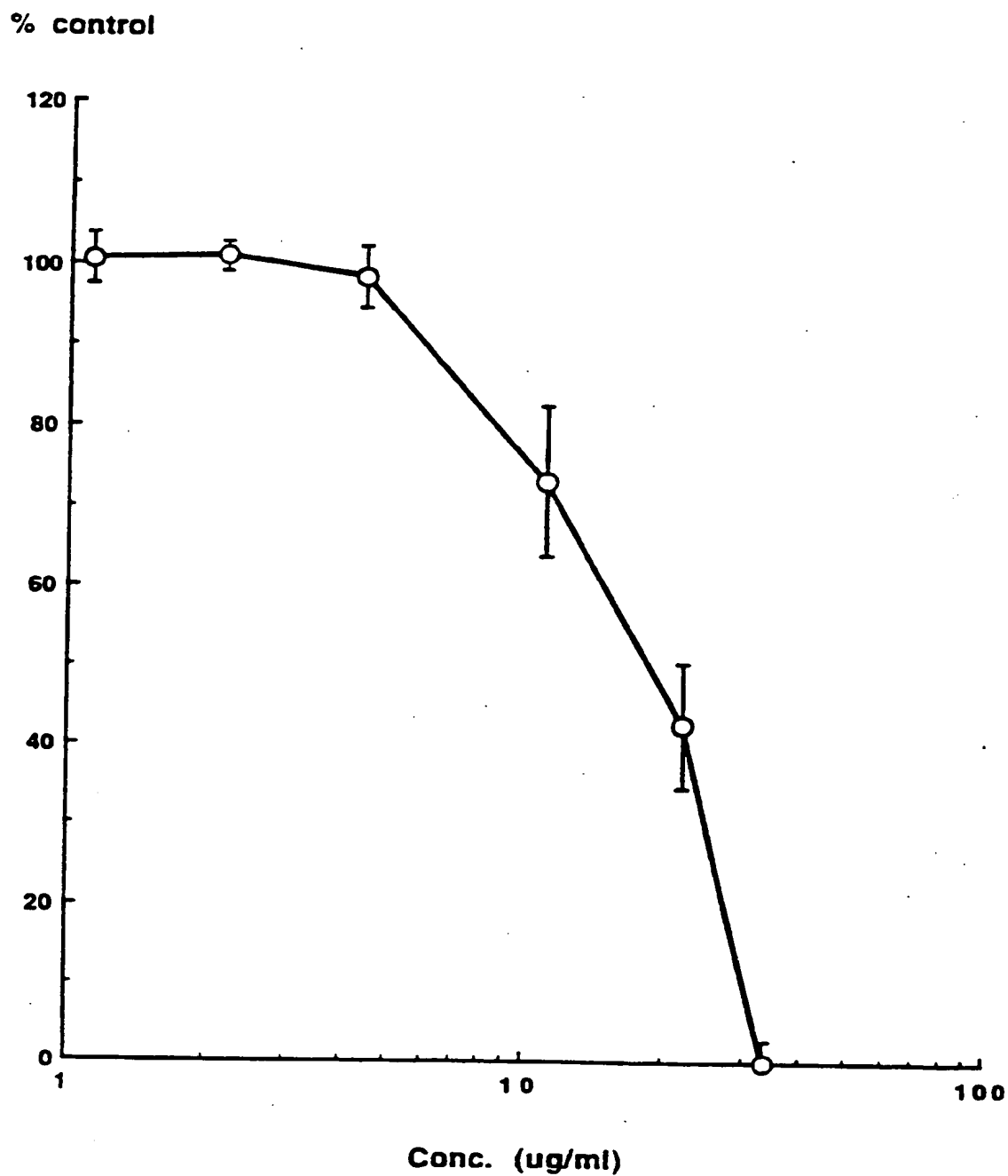


FIG. 3



Effect of trans, trans farnesol on PC3 growth

FIG. 4
SUBSTITUTE SHEET

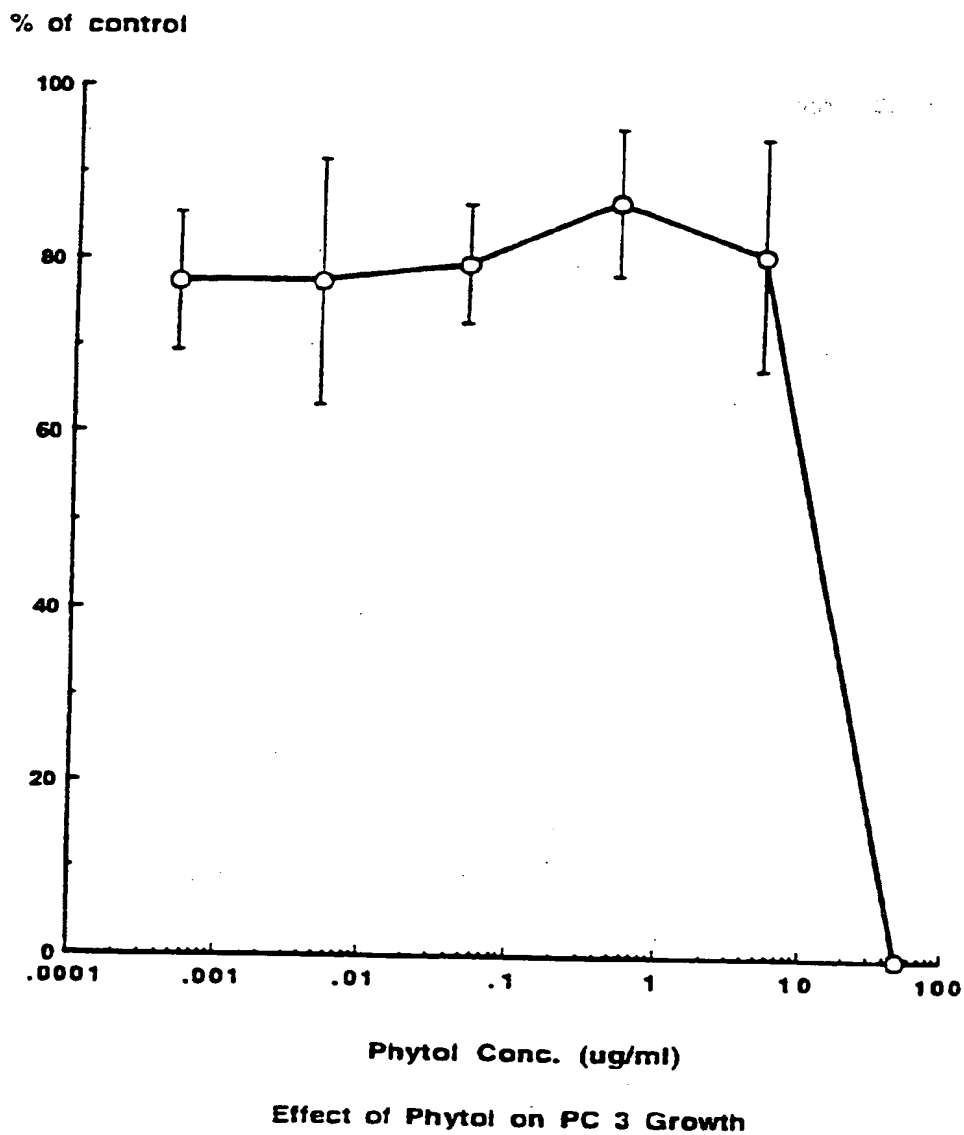


FIG. 5

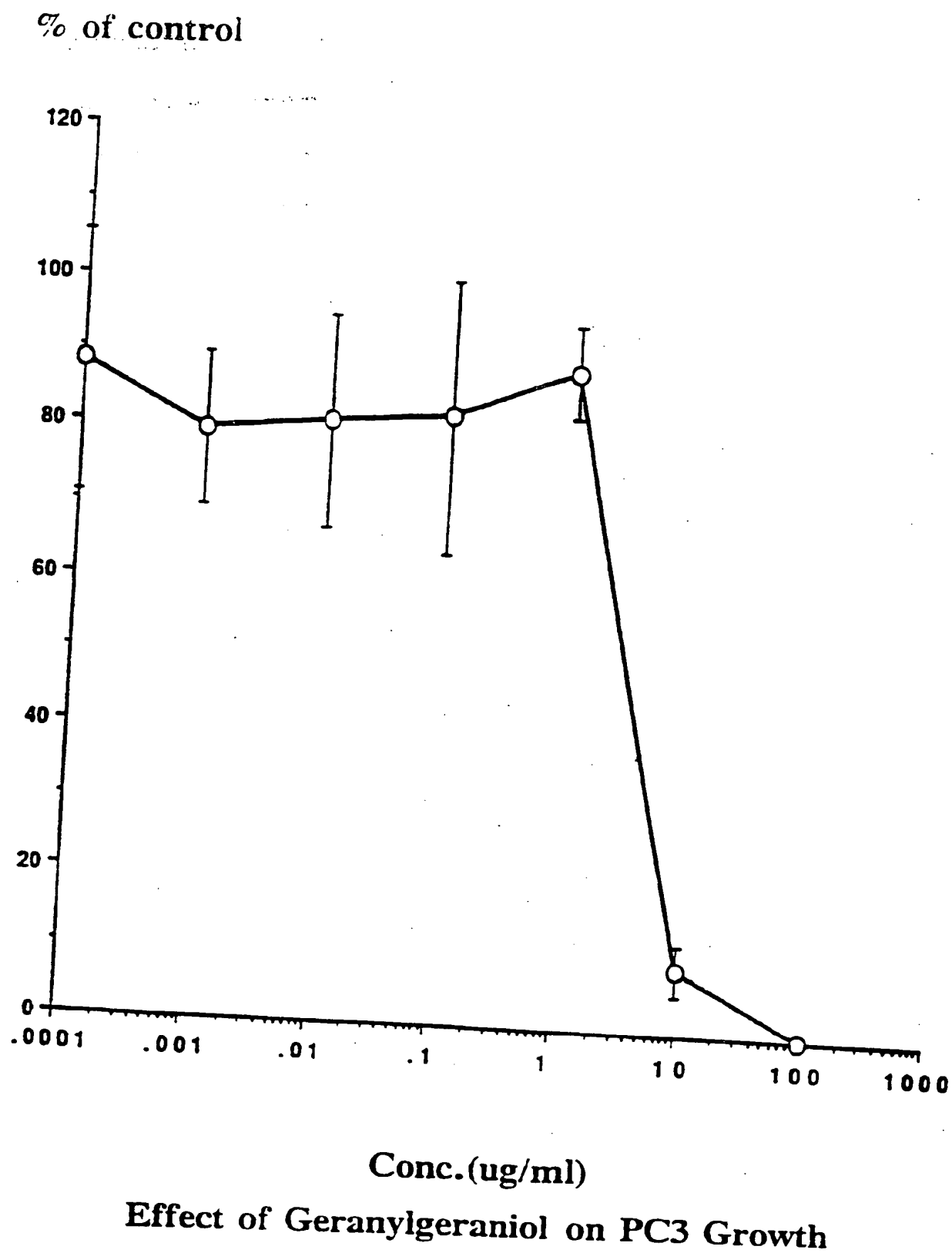


FIG. 6

- 7 / 13

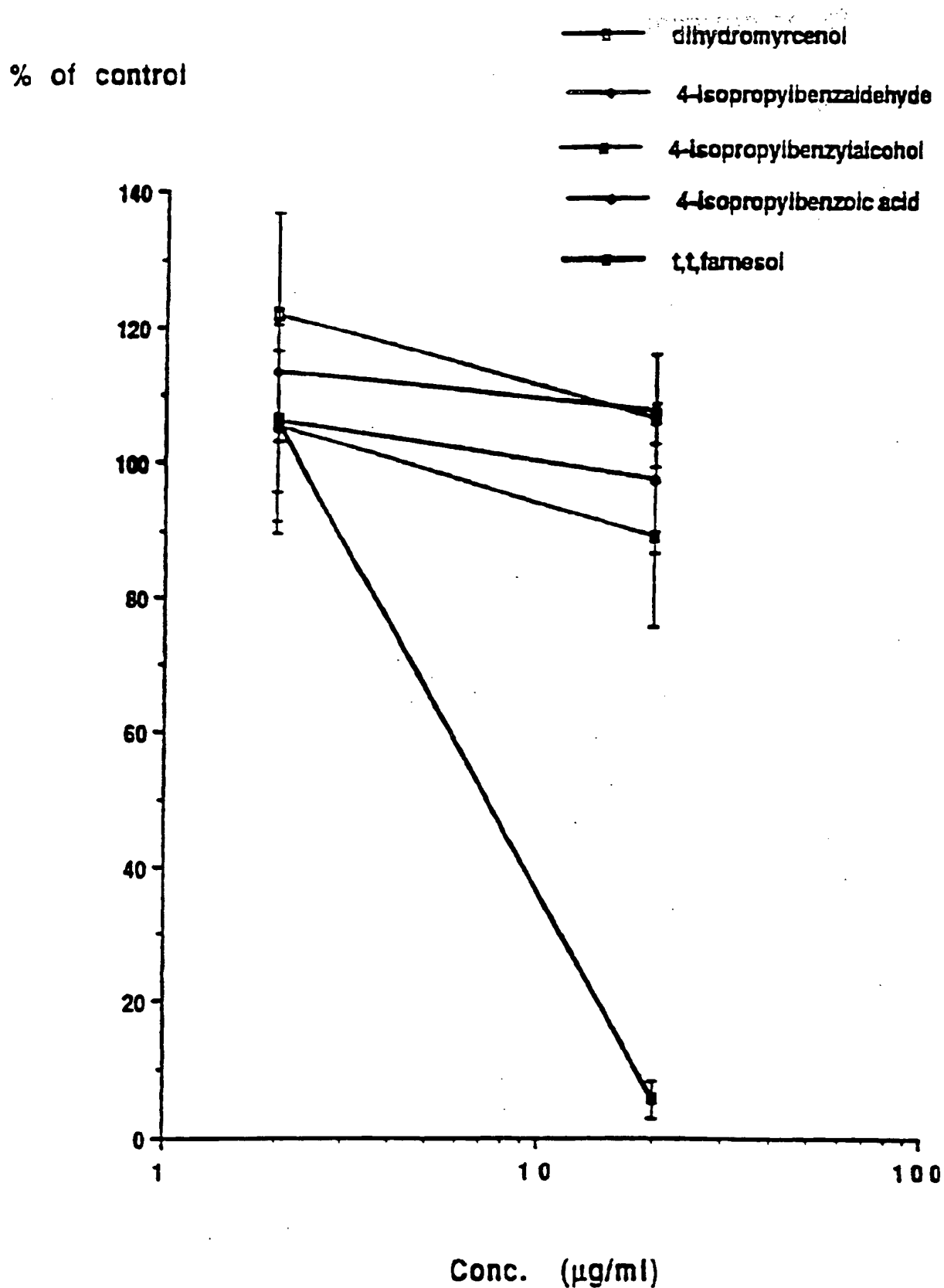


FIG. 7

SUBSTITUTE SHEET

- 8 / 13

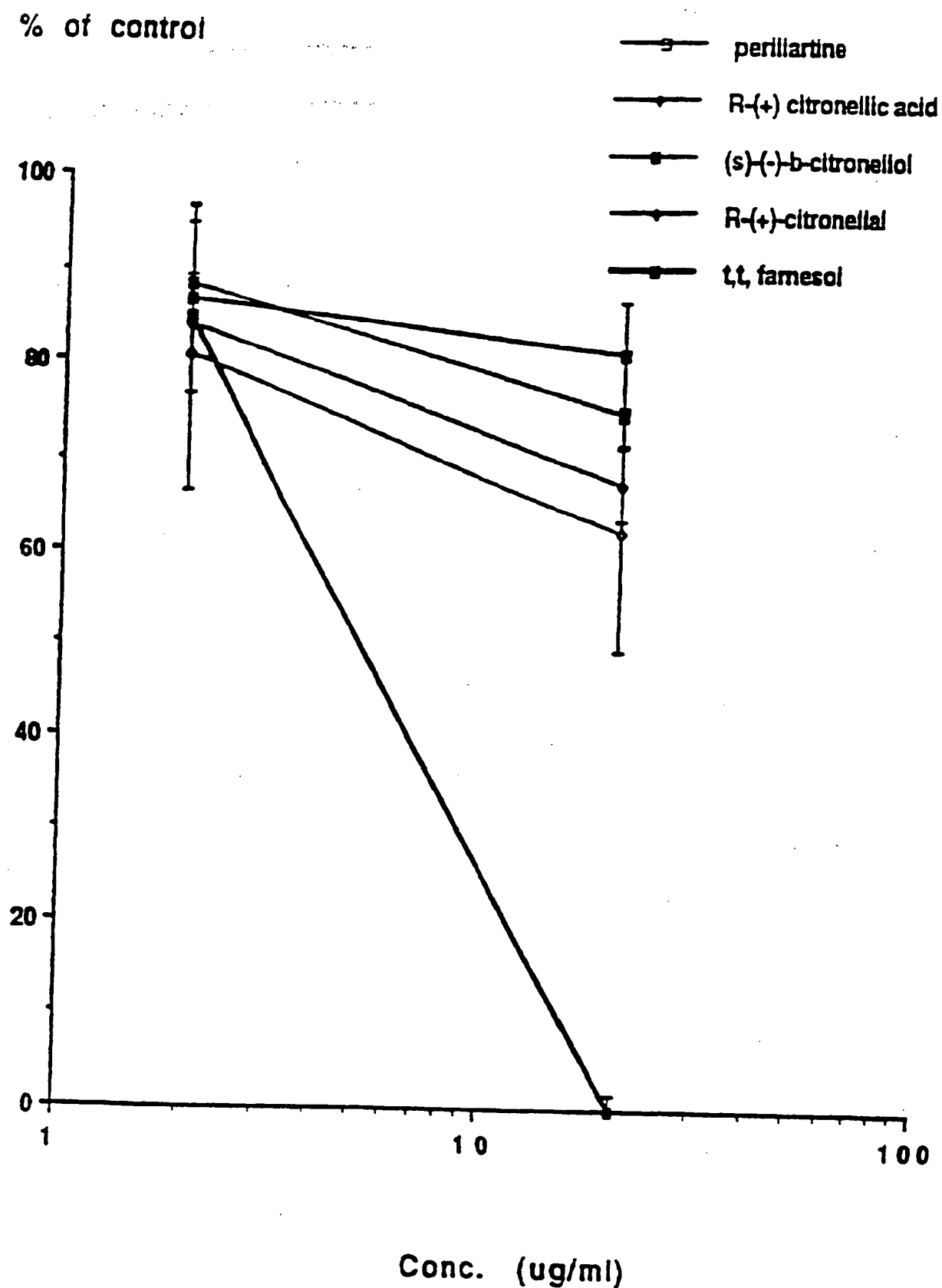


FIG. 8

SUBSTITUTE SHEET

- 9 / 13

% of control

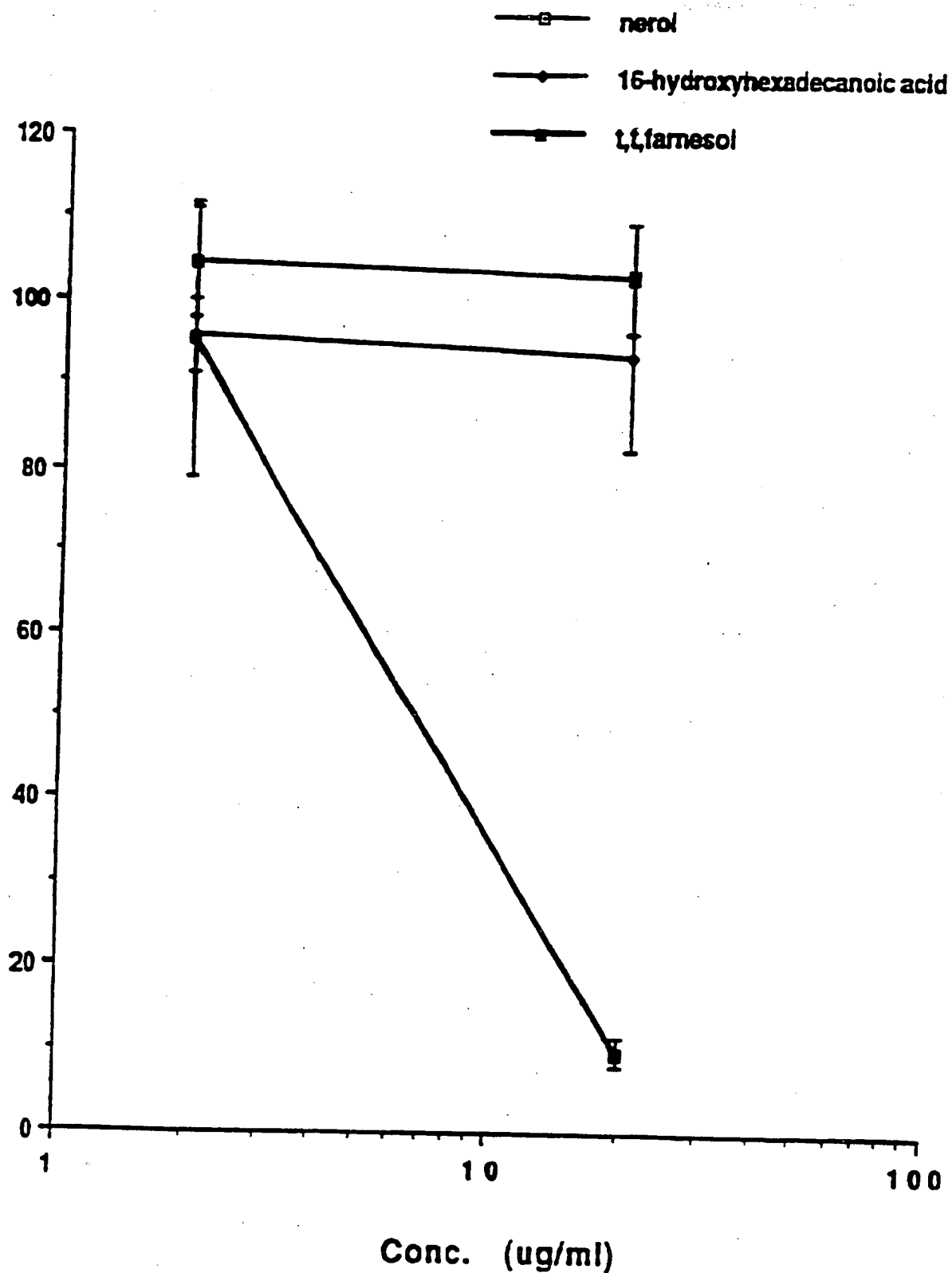


FIG. 9

SUBSTITUTE SHEET

AD5 human osteosarcoma cells radioresistant via activated ras:
Reversal by limonene

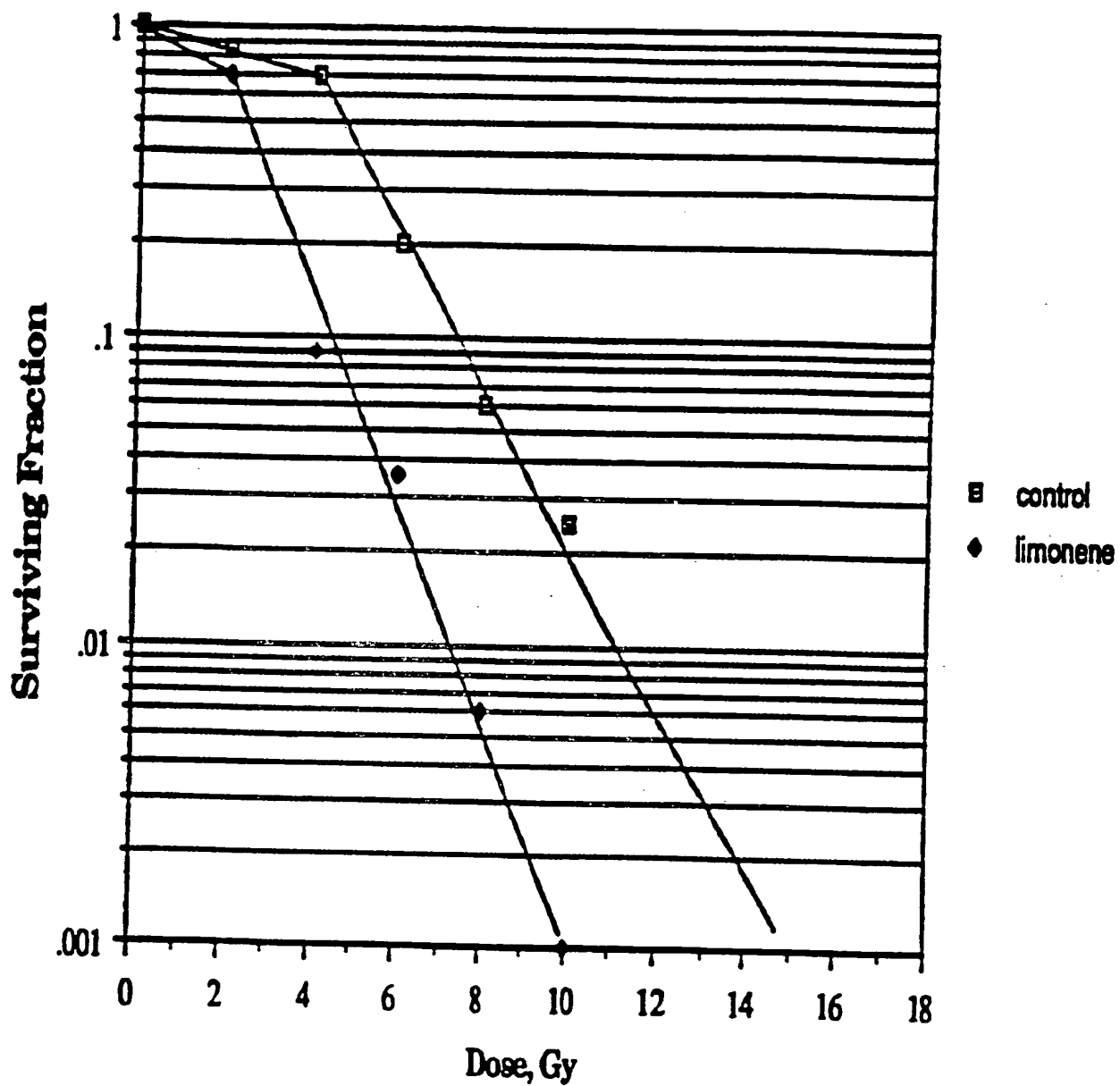


FIG. 10

11 / 13



.3/2 +
 1/1
 + 1/1 +
 1/2 +
 -2.6/1
 /2
 -.75/1 +
 2 +

FIG. 11.

12 / 13

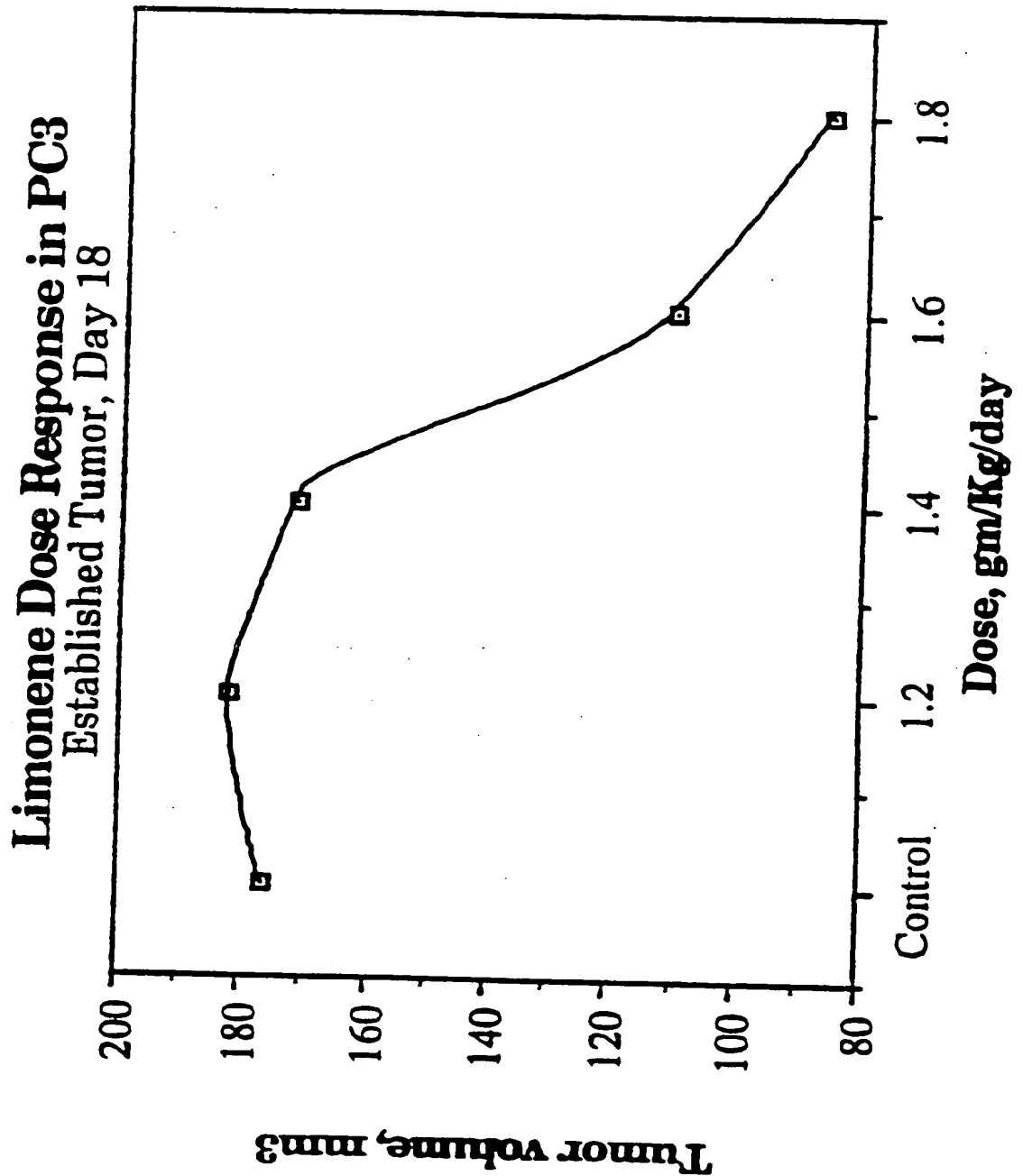


FIG. 12

SUBSTITUTE SHEET

13 / 13

Activity of daily oral phytol

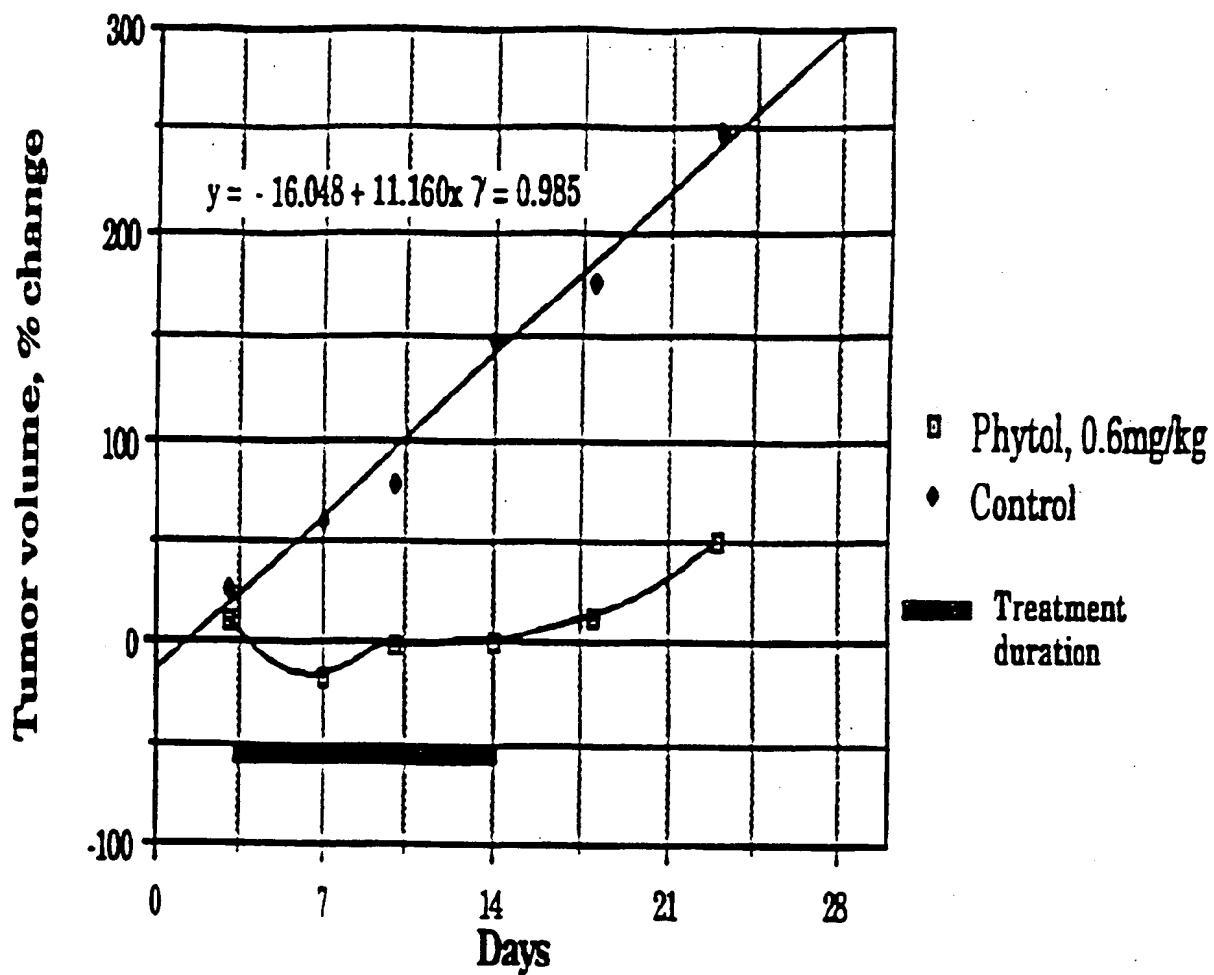


FIG. 13

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

Intel. Application No

PCT/US 94/02340

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/01 A61K31/015 A61K31/045

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 285 302 (HARBOR BRANCH) 5 October 1988 see page 4, line 20 - line 27 see page 7, line 3 - line 4 see claims 1-3,7 ---	1-12, 28-34
X	DATABASE WPI Derwent Publications Ltd., London, GB; AN 88-004865 & JP,A,2 270 544 (RIKAGAKU KENKYUSHO) 24 November 1987 see abstract ---	1-12, 28-34
X	WO,A,92 18465 (THE PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 29 October 1992 see page 1, line 1-14 see page 3, line 13 - line 16 see page 5, line 11 - line 15 --- -/--	1-12, 28-34



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search

15 June 1994

Date of mailing of the international search report

24.06.94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Gerli, P

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/US 94/02340

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages.	Relevant to claim No.
X	EP,A,0 393 973 (LEMON GRASS FOOD CO. LTD.) 24 October 1990 see page 3, line 51 - line 53 see page 3, line 1 - line 8 ---	1-12, 28-34
Y	FR,A,2 036 924 (BARANGER P.) 31 December 1970 ---	1-12, 28-34
Y	DATABASE WPI Derwent Publications Ltd., London, GB; AN 91-167012 & JP,A,3 098 562 (TAIYO) 24 April 1991 see abstract ---	1-12, 28-34
Y	DE,A,33 18 989 (EISAI) 1 December 1983 see page 14, line 27 - line 30 see page 11, line 19 -----	1-12, 28-34

INTERNATIONAL SEARCH REPORT

Intern. application No.

PCT/US94/02340

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 1-34 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Appl. No.

PCT/US 94/02340

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0285302	05-10-88	JP-A- 1019054	23-01-89
WO-A-9218465	29-10-92	US-A- 5202456	13-04-93
		CA-A- 2107790	16-10-92
		EP-A- 0580786	02-02-94
EP-A-0393973	24-10-90	JP-A- 2279629	15-11-90
		CA-A- 2014679	18-10-90
FR-A-2036924	31-12-70	NONE	
DE-A-3318989	01-12-83	JP-C- 1583050	22-10-90
		JP-B- 2006326	08-02-90
		JP-A- 58206517	01-12-83
		JP-C- 1583052	22-10-90
		JP-B- 2006328	08-02-90
		JP-A- 58225014	27-12-83
		JP-C- 1583059	22-10-90
		JP-B- 2006327	08-02-90
		JP-A- 59073513	25-04-84
		JP-C- 1575820	24-08-90
		JP-B- 2000330	08-01-90
		JP-A- 59073533	25-04-84
		AT-B- 389871	12-02-90
		BE-A- 896865	28-11-83
		CH-A- 654823	14-03-86
		FR-A- 2527597	02-12-83
		FR-A- 2532843	16-03-84
		FR-A, B 2532848	16-03-84
		FR-A, B 2532844	16-03-84
		FR-A- 2532845	16-03-84
		FR-A- 2532846	16-03-84
		FR-A- 2532847	16-03-84
		FR-A- 2569108	21-02-86
		GB-A, B 2122610	18-01-84
		GB-A, B 2159054	27-11-85
		GB-A, B 2159709	11-12-85
		GB-A, B 2159055	27-11-85
		GB-A, B 2159710	11-12-85
		GB-A, B 2159711	11-12-85

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 94/02340

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A-3318989		GB-A, B 2159712	11-12-85
		GB-A- 2159713	11-12-85
		NL-A- 8301892	16-12-83
		SE-B- 461650	12-03-90
		SE-A- 8303013	29-11-83
		SE-A- 8801513	22-04-88
		SE-A- 8801514	22-04-88
		SE-A- 8801515	22-04-88
		US-A- 5280048	18-01-94
		US-A- 4624966	25-11-86
